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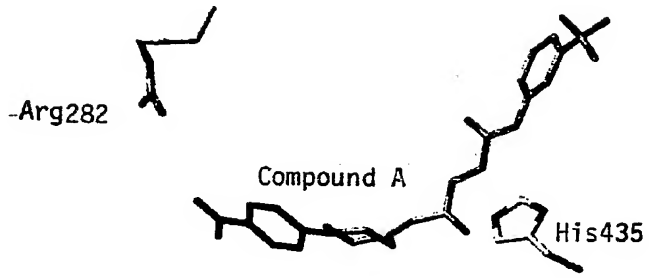

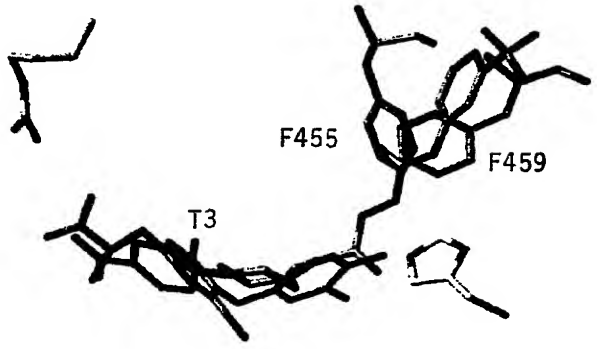

(57) Abstract: Provided are compounds, pharmaceutical compositions, and methods for the synthesis and use thereof that are effective for the modulation or treatment of conditions characterized by overproduction of thyroid hormone, wherein the effective compounds act by antagonizing the effect of thyroid hormone at the receptor level.

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DIVERSE THYROID HORMONE RECEPTOR ANTAGONISTS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit of priority to provisional patent application Serial No. 60/470,749, filed May 14, 2003, which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENTAL SUPPORT

[0002] The research leading to the present invention was supported, at least in part, by National Institute of Health (NIH) Grant Nos. DK16636 and DK059041. Accordingly, the United States Government may have certain rights in the invention.

FIELD OF THE INVENTION

[0003] The invention relates to compounds and pharmaceutical compositions, and to the uses thereof, that are effective for treating conditions characterized by overproduction of thyroid hormone.

BACKGROUND OF THE INVENTION

[0004] Overproduction of thyroid hormone (hyperthyroidism or thyrotoxicosis) is an extremely common clinical entity caused by a number of different pathological conditions of the thyroid gland. Approximately 0.5% of women will experience some clinical manifestation of hyperthyroidism in their lifetime (a frequency 3 to 5 times higher than that occuring in men), with potentially life-threatening effects on the cardiovascular system, including cardiac arrhythmias, heart failure, angina and myocardial infarction, particularly in the elderly[1-3].

[0005] The treatment of hyperthyroidism has essentially remained unchanged for the past thirty years, and includes the use of radioactive iodine, surgery, or the use of anti-thyroid drugs, such as propylthiouracil, that inhibit thyroid hormone synthesis by blocking the iodination of thyroglobulin[1-3]. Each approach has its own intrinsic limitations and/or side effects. Propylthiouracil and related drugs, which block thyroid hormone synthesis, act slowly and can take up to six to eight weeks to fully deplete the thyroid gland and intrathyroidal stores of iodinated thyroglobulin, during which time hyperthyroidism can have severe consequences in certain individuals. Radiochemical destruction of thyroid tissues by iodine may require four to six months

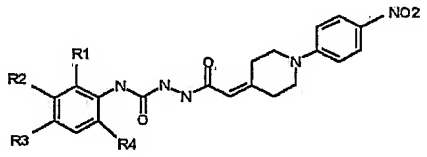
to be fully effective while surgical thyroidectomy must be preceded with anti-thyroid drugs to prevent life threatening complications such as thyroid storm.

[0006] The identification of thyroid hormone receptor ("TR") antagonists could play an important role in the future treatment of hyperthyroidism. Such molecules would act rapidly by directly antagonizing the effect of thyroid hormone at the receptor level, a significant improvement for individuals with hyperthyroidism who require surgery, have cardiac disease, or life threatening thyrotoxic storm.

SUMMARY OF THE INVENTION

[0007] The present invention concerns the usage of ligands having the effect of antagonizing TR as pharmaceutical agents. The compounds of interest are ligands capable of bonding to TR. These compounds and pharmaceutical compositions containing them are useful for the treatment of conditions such as hyperthyroidism which are characterized by an overproduction of TR by the thyroid gland. Additionally, the invention includes a method for the computer based screening, optimization, in vitro testing, and synthesis of novel compounds having TR antagonist activity using a library that may include commercially available starting compounds.

[0008] In a first aspect, the invention provides pharmaceutical compositions that are capable of antagonizing TR, that have as an active ingredient a compound or compounds that have the structure of Formula I:



I

In Formula I:

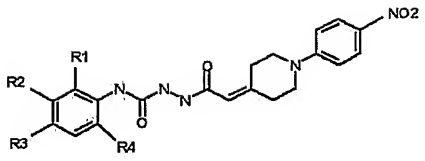
R_1 is $CH(CH_3)_2$, CH_2CH_3, CH_3, or H;

R_2 is CF_3, CH_3, F, or H;

R_3 is F, CH_3, OCH_3, CF_3, or H; and

R_4 is CH_3, OCH_3, or H.

[0009] In a second aspect, the invention concerns a particular group of compounds according to Formula I that have been identified and synthesized herein, and that are ligands having TR antagonist activity that and may be defined by Formula I:



I

wherein

R_1 is $CH(CH_3)_2$, CH_2CH_3, CH_3, or H;

R_2 is CF_3, CH_3, F, or H;

R_3 is F, CH_3, OCH_3, CF_3, or H; and

R_4 is CH_3, OCH_3, or H

provided that

when R_2 is CF_3, R_1, R_3 and R_4 are H;

when R_1 is $CH(CH_3)_2$, R_2 and R_3 are H and R_4 is either CH_3 or H;

when R_1 is CH_2CH_3, R_2, R_3, and R_4 are H;

when R_2 is CH_3, R_1 and R_3 are H and R_4 is OCH_3;

when R_2 is F, R_3 is also F and R_1 and R_4 are H;

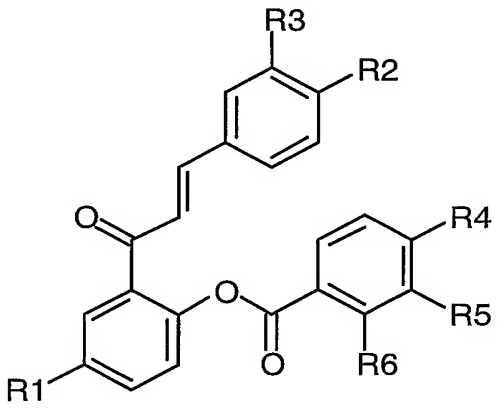
when R_3 is F, R_2 is also F and R_1 and R_4 are H;

when R_1 is CH_3, R_3 and R_4 are also CH_3 and R_2 is H;

when R_3 is OCH_3, R_1, R_2, and R_4 are H;

when R_3 is CF_3, R_1, R_2, and R_4 are H.

[0010] In a third aspect, the invention concerns derivatives of a certain compound which is designated herein Compound F, and methods for the synthesis thereof, and the sythesis of its derivatives in turn, whereby said derivatives of Compound F comprise a class of compounds that may be generally represented by Formula II



Formula II

wherein

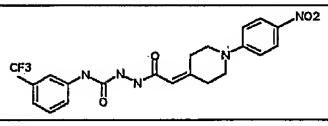
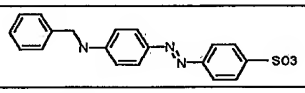
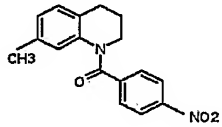
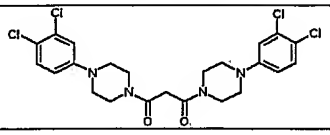
R1 is F;
R2 may be Cl, OCH_3 or F;
R3 may be H or OCH_3;
R4 may be H or NO_3;
R5 may be H, OCH_3 or NO_2; and
R6 may be H or OCH_3.

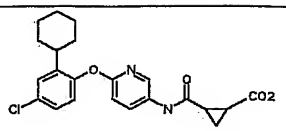
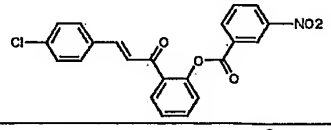
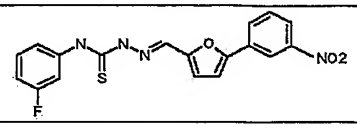
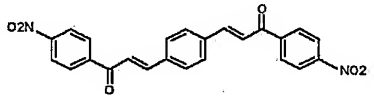
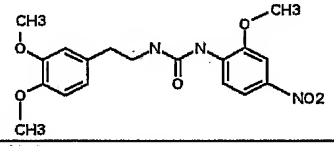
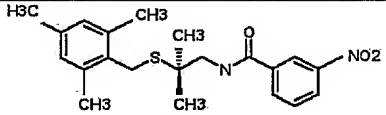
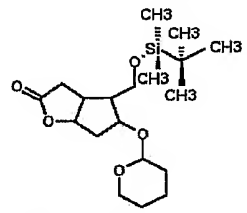
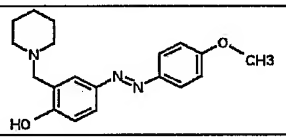
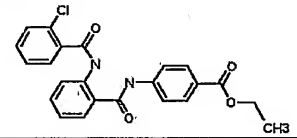
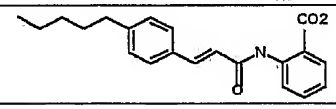
In a preferred embodiment, the compounds prepared in accordance with Formula II may be selected from the following:

R1 = F, R2 = Cl, R3 = H, R4=H, R5=NO_2, R6=H
R1 = F, R2 = Cl, R3 = H, R4=H, R5=H, R6=OCH_3
R1 = F, R2 = OCH_3, R3 = OCH_3, R4=H, R5=NO_2, R6=H
R1 = F, R2 = Cl, R3 = H, R4=NO_2, R5=H, R6=H
R1 = CH_3, R2 = F, R3 = H, R4=H, R5= OCH_3, R6=H

[0011] In a fourth aspect, the invention provides a method for the identification, screening, optimization of selectivity of, and synthesis of compounds capable of antagonizing the effects of TR, wherein the method comprises the steps of

i) selecting a compound, such as a compound selected from the group consisting of

	Structure
Compound A	
Compound B	
Compound C	
Compound D	

Compound E	
Compound F	
Compound G	
Compound H	
Compound I	
Compound J	
Compound K	
Compound L	
Compound M	
Compound N	

ii) generating a virtual library of derivatives of the compound chosen in step i);

iii) screening said library in silico;

iv) chemically synthesizing at least one compound screened in iii); and

v) testing in vitro at least one compound synthesized in iv).

[0012] In a fifth aspect, the invention provides original ligands with TR antagonist activity in the □M range and sub-□M range.

[0013] In a sixth aspect, the invention provides pharmaceutical compositions comprising one or more compounds of the invention, effective for the treatment of conditions such as hyperthyroidism and thyrotoxicosis characterized by overproduction of thyroid hormone wherein the compositions act by antagonizing TR.

[0014] In a seventh aspect, the invention provides methods for modulation a process mediated by thyroid hormone nuclear receptors by administering to a human a compound or composition according to the invention that is capable of antagonizing TR.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1A is a predicted conformation of Compound A bound to the TRβ ligand-binding pocket. A hydrogen bond between His 435 and a carbonyl oxygen of Compound A and possibly between Arg 282 and a nitro-oxygen of Compound A constitute the only polar interactions. All other contacts are hydrophobic (not shown for clarity).

[0016] Figure 1B shows a predicted conformation of Compound A superimposed with the crystal structure of T3 bound to active TR, and clashing with the active conformation of helix H12.

[0017] Figure 2 is a graph showing inhibition of [^{125}I]T3 binding to TR by Compound A in intact cells. The GH4C1 pituitary cell line, which contains endogenous TRs (TRα, TRβ1, and TRβ2), was incubated with 0.1 nM [^{125}I]T3 alone and with the indicated concentrations on unlabeled T3 and Compound A. After incubation for 60 min. at 37°C, the cells were chilled, washed, and the nuclei isolated. The results indicate the inhibition of binding of [^{125}I]T3 by T3 and Compound A.

[0018] Figure 3 is a comparison of the antagonist activity of Compound A and two of its derivative compounds (A_1 and A_3) identified through in silico virtual library screening in accordance with the invention.

[0019] Figure 4 is a graph showing the inhibition of T3-mediated co-activator recruitment to TR by compounds A_1, A_3, and A in vitro. Approximately 2.5-5 x 10^4 cpm of ^{35}S-labeled TRα α(20 fmol) in 2 µl of lysate was incubated with 500 ng of GST fused

to the receptor interaction region of the co-activator NRC (NRC15) immobilized on glutathione-agarose beads. The samples were also incubated for 15 min at room temperature with 2 μM of A_1 or A_3 or 5 μM of Compound A in binding buffer. The samples were then chilled on ice and incubated with 1 nM T3 for an additional 60 min at 4°C. Control samples contained no T3 or antagonists, or received only T3. The beads were washed and the bound ^{35}S-TRα electrophoresed in a 10% SDS-gel followed by analysis and quantitation of the amount of ^{35}S-TRα bound using a Molecular Dynamics Phosphorimager and ImageQuant software. The percent inhibition of T3-mediated binding of ^{35}S-TRα to GST-NRC15 by compounds A, A_1, and A_3 was determined after subtracting the amount of ^{35}S-TRα bound to GST-NRC15 in the absence of T3.

[0020] Figure 5 is a graph of the standard UV absorption-concentration correlation for compound A4 concentration in mice serum, as discussed in Example 4.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0021] Definitions. Unless otherwise provided herein:

[0022] "pharmaceutically acceptable" means that the carrier, diluent, vehicle excipients, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof;

[0023] "pharmaceutically acceptable salts" of the compounds of this invention may be formed of the compound itself, prodrugs, e.g. esters, isomers and the like, and include all of the pharmaceutically acceptable salts which are most often used in pharmaceutical chemistry; for example, salts may be formed with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, carboxylic acids, sulfonic acids including such agents as naphthalenesulfonic, ethanesulfonic, hydroxyethanesulfonic, methanesulfonic ("mesylate"), benzenesulfonic ("besylate") and toluenesulfonic acids, e.g., p-toluenesulfonic ("tosylate"), sulfuric acid, nitric acid, phosphoric acid, tartaric acid, pyrosulfuric acid, metaphosphoric acid, succinic acid, formic acid, phthalic acid, malic acid, maleic acid, lactic acid, ascorbic acid, glycollic acid, gluconic acid, mandelic acid, glutamic acid, aspartic acid, fumaric acid, pyruvic acid, phenylacetic acid, pamoic acid, nicotinic acid, and the like; suitable pharmaceutically acceptable salts also include alkali metal salts (e.g. sodium, potassium salts), alkaline earth metal salts (e.g. magnesium, calcium salts), amine

salts (e.g. ammonium, alkylammonium, dialkylammonium, trialkylammonium, tetraalkylammonium, diethanolaminium, tri-ethanolaminium and guanidinium salts); preferred salts include salts of organic acids selected from formic, acetic, trifluoroacetic, propionic, benzoic, citric, maleic, tartaric, methanesulfonic, benzenesulfonic or toluenesulfonic, salts of inorganic acids selected from hydrochloric, hydrobromic, sulfuric or phosphoric, amino acids selected from aspartic and glutamic, and salts of sodium and potassium;

[0024] a "prodrug" is a drug precursor which, following administration, releases the drug in vivo via some chemical or physiological process (e.g., a prodrug on being brought to the physiological pH or through enzyme action is converted to the desired drug form);

[0025] "treating," "treat" or "treatment" includes, inter alia, preventative (e.g., prophylactic), palliative and curative treatment.

[0026] The invention relates to the discovery of original ligands with TR antagonist activity in the □M range and sub-□M range, to pharmaceutical compositions containing such compounds, and to the uses thereof. The activity of the thyroid hormones, L-thyroxin (T4) and L-triiodothyronine (T3), is mediated by the thyroid hormone receptor ("TR") [45,50,51]. The TRs are members of the nuclear hormone receptor (NR) superfamily that also includes receptors for steroid hormones, retinoids, and 1,25-dihydroxy-vitamin D3[5-7]. These receptors are transcription factors that can regulate expression of specific genes in various tissues, and are targets for widely used drugs, such as tamoxifen, an estrogen receptor (ER) partial antagonist, flutamide, an anti-androgen, or rosiglitazone, a peroxisome proliferator activated receptor-□ (PPAR□) agonist (Dees 1998) (Olefsky 2000).

[0027] Several different isoforms of TR (TR-□1, TR-□1 and TR-□2) are differentially expressed in various tissues and have been described (Lazar 1993). Gene knockout studies in mice indicate that the TR□ isoforms plays a role in the development of the auditory system and in the negative feedback of thyroid stimulating hormone ("TSH") by T3 in the pituitary (Forrest 1996, Weiss 1997), while TR□ modulates the effect of thyroid hormone on calorigenesis and on the cardiovascular system (Wikstrom 1998).

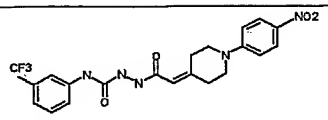
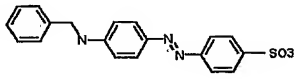
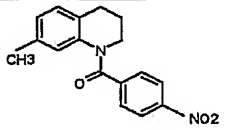
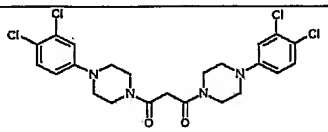
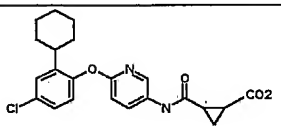
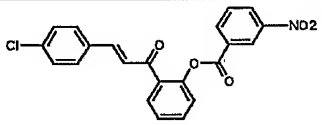
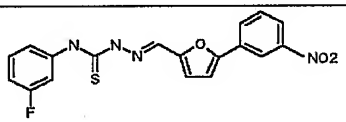
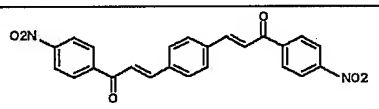
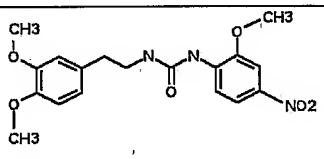
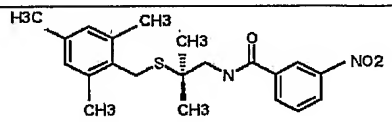
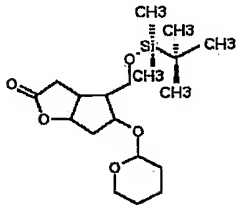
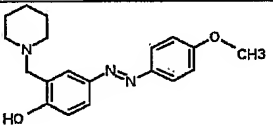
[0028] In a preferred embodiment, compounds according to the invention act to antagonize TR. Previous expertise in the structure/function of NRs facilitated the construction of a TR model in its antagonist-bound conformation (see Figs. 1A and 1B).

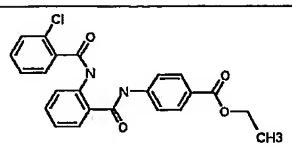
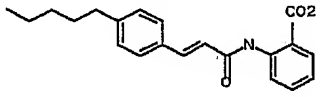
[0029] In a preferred embodiment, antagonist candidate molecules are selected by in silico screening from a large library that may include known compounds. Each ligand of the Available Chemicals Directory ("ACD") of over 240,000 commercially available chemical structures was automatically docked into the model of the TR antagonist binding pocket. The unexpected chemical diversity of active molecules identified underlines the power of the receptor-based rational lead drug-discovery approach. The best scoring compounds were then further energy-minimized using a full atom representation of the receptor according to a double-scheme Monte-Carlo energy minimization procedure with both flexible ligand and flexible receptor side-chains. Previous achievements demonstrate that this strategy can successfully identify receptor-specific ligands with appropriate biological systems and robust modeling tools (Schapira 2000, Filikov 2000, Schapira 2001, Abagyan 2002). As a non-limiting example, the C-terminal H12 helix of TR was docked onto the hydrophobic co-activator recruitment site of the receptor, and the energy of the system was minimized in the internal coordinates space by an extensive Monte Carlo simulation, which may be (and this illustration was) performed with Molsoft's ICM technology (ICM 2.8 Manual).

[0030] Each compound was scored according to its fit with the TR□ receptor model, taking into account continuum as well as discrete electrostatics, hydrophobicity, and entropy parameters. Fourteen structurally diverse TR antagonists were identified in this way. One optimization cycle, based on one of the 14 active structures, allowed improvement of the affinity for the receptor. The 14 known molecules displaying TR antagonist activity are listed below in Table I in the order of apparent efficacy against an agonist concentration of 8 nM T3. The unexpected chemical diversity of active molecules identified underlines the power of the receptor-based rational lead drug discovery approach.

TABLE 1

		Concentration (□M)	Inhibition (%)

Compound A		20	90
Compound B		20	80
Compound C		20	66
Compound D		20	62
Compound E		20	55
Compound F		20	53
Compound G		20	53
Compound H		20	50
Compound I		20	49
Compound J		20	42
Compound K		20	36
Compound L		20	36

Compound M		20	34
Compound N		4 (Toxic at 20 □M)	33

[0031] In a preferred embodiment, Compound A, chosen for its large (90%) inhibition at a concentration of 20μM is further derivatized by the synthetic schemes outlined below. Referring to Figure 1A, the predicted conformation of antagonist candidate Compound A bound to TR□ ligand-binding pocket is shown. A hydrogen bond between His435 and a carbonyl oxygen of Compound A and possibly between Arg 282 and a nitro-oxygen of Compound A would be the only polar interactions. All other contacts would be hydrophobic (not shown for clarity) highlighting the antagonistic properties of Compound A at the receptor site. Figure 1B shows that Compound A would superimpose with the crystal structure of T3 bound to active TR and would clash with the active conformation of helix H12.

[0032] Eight compounds, Compounds A_1-A_8, all derivatives of Compound A, were actually synthesized and tested in vitro. The calculated score, corresponding rank, structure and activity for each compound, represented as a species of a genus represented by Formula I, are listed below in Table 2. The best two inhibitors were among the top 4 scoring compounds (A_1 and A_3 respectively), while the two less active molecules were the worst scoring ones (A_7 and A_8 respectively). One derivative (A_3) reached IC-50 in the nanomolar range (0.75 □M)

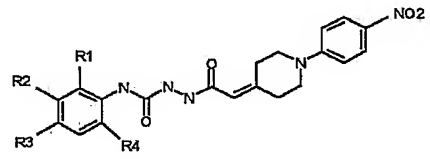
Formula I 

TABLE 2

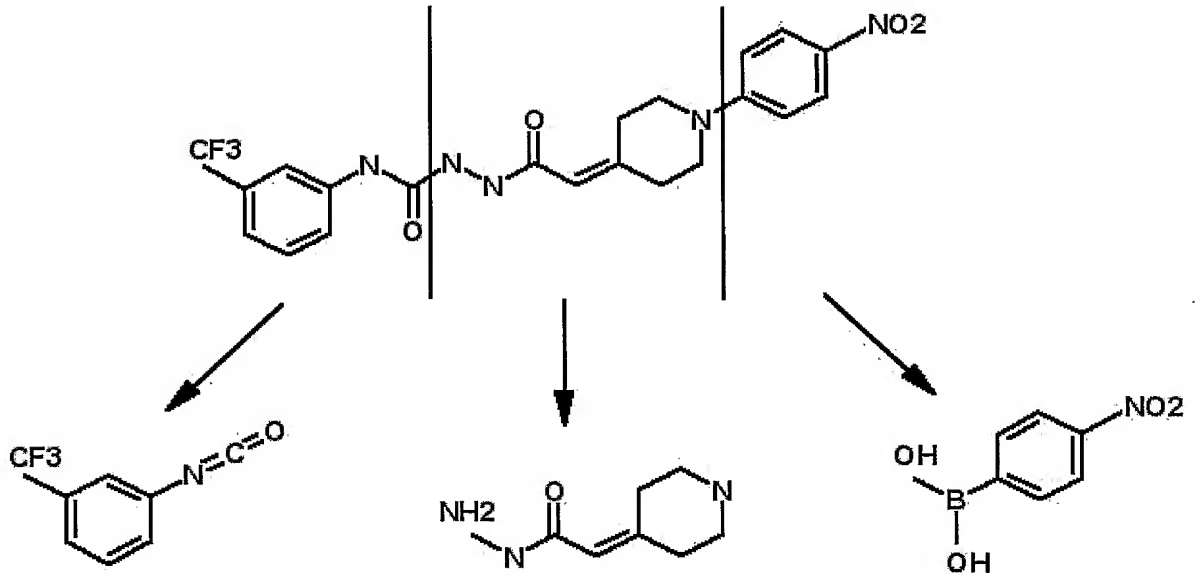
Name	Score	Rank (predict.)	Structure	Conc. (□M)	Inhib. (%)
Compound A	- 32.86	-	R2: -CF3	1.5 □M 5 □M	50 80
Compound A_1	- 35.98	1	R1: -CH(CH3)2	2.5 □M 5 □M	50 76

Compound A_2	- 34.39	3	R1: -CH2-CH3	5 □M	28
Compound A_3	- 33.85	4	R1: -CH(CH3)2 R4: -CH3	0.75 □M 5 □M	50 84
Compound A_4	- 33.80	5	R2: -CH3 R4: -OCH3	5 □M	21
Compound A_5	- 31.82	12	R2: F R3:F	5 □M	67
Compound A_6	- 30.87	19	R1: -CH3 R3: -CH3 R4: -CH3	5 □M	20
Compound A_7	- 29.09	37	R3: -OCH3	5 □M	10
Compound A_8	- 27.94	57	R3: -CF3	5 □M	10

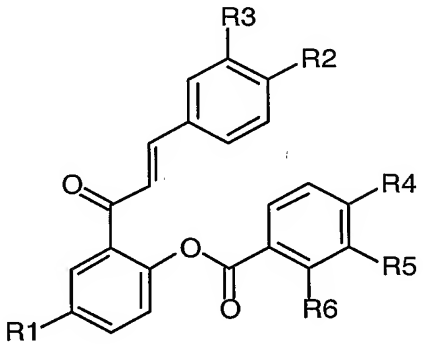
[0033] As disclosed herein, a compound within the scope of Formula I shall at all times be understood to include all active forms of such compounds, including, for example, the free form thereof, e.g., the free acid or base form and also, all prodrugs, polymorphs, hydrates, solvates, and the like, and all pharmaceutically acceptable salts as described above. It will also be appreciated that suitable active metabolites of compounds within the scope of Formula I, in any suitable form, are also included herein.

[0034] Moreover, certain compounds suitable for use in the present invention such as, for example, certain compounds of Formula I may have asymmetric centers and therefore exist in different enantiomeric forms. All suitable optical isomers and stereoisomers of such compounds, and mixtures thereof, are considered to be within the scope of the invention. With respect to such compounds, the present invention includes the use of a racemate, a single enantiomeric form, a single diastereomeric form, or mixtures thereof, as suitable. Moreover, such compounds may also exist as tautomers. Accordingly, the present invention relates to the use of all such suitable tautomers and mixtures thereof.

[0035] In a preferred embodiment of the invention, the scheme shown below is employed to generate a virtual library focused on Compound A, in which Compound A is divided into three structural units that can be derivatized independently with commercially available building blocks".



[0036] In another embodiment, Compound F is further derivativized with commercially available building blocks to obtain a class of compounds represented in general form by Formula II below.



Formula II

Where

R1 = F, R2 = Cl, R3 = H, R4=H, R5=NO_2, R6=H	Ki=10μM
R1 = F, R2 = Cl, R3 = H, R4=H, R5=H, R6=OCH_3	Ki=10μM
R1 = F, R2 = OCH_3, R3 = OCH_3, R4=H, R5=NO_2, R6=H	Ki=5μM
R1 = F, R2 = Cl, R3 = H, R4=NO_2, R5=H, R6=H	Ki=10μM
R1 = CH_3, R2 = F, R3 = H, R4=H, R5= OCH_3, R6=H	Ki=10μM

[0037] In a preferred embodiment, the invention comprises pharmaceutical compositions having synthesized compounds of Table 2, or other novel derivatives of the compounds of Table I, synthesized as above described, or prodrugs, isomers or pharmaceutically acceptable salts thereof, as their active ingredients.

Pharmaceutical compositions according to the invention preferably comprise a suitable amount of at least one compound, prodrug, isomer or pharmaceutically acceptable salt of this compound, (i.e. an amount sufficient to provide the desired dosage) along with a pharmaceutically acceptable vehicle, carrier or diluent.

[0038] The compounds, prodrugs, isomers and pharmaceutically acceptable salts of this invention can be administered in a wide variety of different dosage forms, i.e., they may be combined with various pharmaceutically acceptable inert carriers in any suitable form. Such carriers include solid diluents or fillers, sterile aqueous media and various non-toxic organic solvents. The pharmaceutical compositions can be formulated to contain a daily dose, or a convenient fraction of a daily dose, in a dosage unit, which may be a single tablet or a capsule or a convenient volume of a liquid.

[0039] Any suitable route of administration may be used in the present invention. It is usually preferred to administer the compounds, prodrugs, isomers and pharmaceutically acceptable salts of this invention orally for reasons of convenience; however, they may be administered, for example, percutaneously, or as suppositories for absorption by the rectum, as desired in a given instance. As described above, the administration may be carried out in single or multiple doses, as appropriate.

[0040] All of the usual types of pharmaceutical compositions may be used in the present invention, including tablets, lozenges, hard candies, chewable tablets, granules, powders, sprays, capsules, pills, microcapsules, solutions, parenteral solutions, troches, injections (e.g., intravenous, intraperitoneal, intramuscular or subcutaneous), suppositories, elixirs, syrups and suspensions.

[0041] For parenteral administration, the compounds, prodrugs, isomers and pharmaceutically acceptable salts of this invention may be used as solutions in sesame or peanut oil, or as aqueous solutions (e.g., aqueous propyleneglycol), as the case may be, and they are best used in the form of a sterile aqueous solution which may contain other substances; for example, enough salts or glucose to make the solution isotonic, the pH of the solution being suitably adjusted and buffered, where necessary, and surfactants such as, for example, hydroxypropylcellulose. Such oily solutions are suitable for intra-articular, intramuscular and subcutaneous injection purposes. Such aqueous solutions are suitable for intravenous injection

purposes.

[0042] The compounds, prodrugs, isomers and pharmaceutically acceptable salts of this invention may also be administered topically and this may be done by way of, e.g., creams, jellies, salves, lotions, gels, pastes, ointments, and the like, in accordance with standard pharmaceutical practice. The compounds, prodrugs, isomers and pharmaceutically acceptable salts of this invention of the present invention may also be administered transdermally (e.g., through the use of a patch). Any suitable formulation for transdermal application comprising a compound of the present invention may be employed and such formulations would generally also contain a suitable transdermal carrier, e.g., an absorbable pharmacologically acceptable solvent to promote and assist passage of the compounds through the subject's skin. For example, suitable transdermal devices may comprise the form of a bandage having a backing member and a reservoir containing the subject compound. Such bandage-type transdermal devices may further include suitable carriers, rate-controlling barriers, and means for securing the transdermal device to the subject's skin.

[0043] In general, all of the pharmaceutical compositions are prepared according to methods usual in pharmaceutical chemistry. As will be described in detail hereinbelow, the pharmaceutical compositions can be prepared by methods commonly employed using conventional, organic or inorganic additives, such as an excipient (e.g., sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, talc, calcium phosphate or calcium carbonate), a binder (e.g., cellulose, methylcellulose, hydroxymethylcellulose, polypropylpyrrolidone, polyvinylpyrrolidone, gelatin, gum arabic, polyethyleneglycol, sucrose or starch), a disintegrator (e.g., starch, carboxymethylcellulose, hydroxypropylstarch, low substituted hydroxypropylcellulose, sodium bicarbonate, calcium phosphate, or calcium citrate), a lubricant (e.g., magnesium stearate, light anhydrous silicic acid, talc or sodium lauryl sulfate), a flavoring agent (e.g., citric acid, menthol, glycine or orange powder), a preservative (e.g., sodium benzoate, sodium bisulfite, methylparaben or propylparaben), a stabilizer (e.g., citric acid, sodium citrate or acetic acid), a suspending agent (e.g., methylcellulose, polyvinylpyrrolidone, or aluminum stearate), a dispersing agent (e.g., hydroxypropylmethylcellulose), a diluent (e.g., water), a coloring agent, an emulsifying agent, and a base wax (e.g., cocoa butter, white petrolatum or polyethylene glycol).

[0044] Any of the compounds, prodrugs, isomers or pharmaceutically acceptable salts of this invention may be readily formulated as tablets, capsules, and the like. It is preferable to prepare solutions from water-soluble salts, such as the hydrochloride salt.

[0045] Capsules can be prepared by mixing a compound, prodrug, isomer or pharmaceutically acceptable salt of the invention with a suitable diluent and filling the proper amount of the mixture in capsules. The usual diluents include inert powdered substances such as starch of many different kinds, powdered cellulose, especially crystalline and microcrystalline cellulose, sugars such as fructose, mannitol and sucrose, grain flours and similar edible powders.

[0046] Tablets can be prepared by direct compression, by wet granulation, or by dry granulation. Their formulations usually incorporate diluents, binders, lubricants and disintegrators as well as a compound, prodrug, isomer or pharmaceutically acceptable salt of this invention. Common diluents include, for example, various types of starch, lactose, mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives may also be used. Common tablet binders include substances such as starch, gelatin and sugars such as lactose, fructose, glucose and the like. Natural and synthetic gums are also convenient, including acacia, alginates, methylcellulose, polyvinylpyrrolidine and the like. Polyethylene glycol, ethylcellulose and waxes can also serve as binders.

[0047] A lubricant is generally necessary in a tablet formulation to prevent the tablet and punches from sticking in the die. The lubricant is chosen from such slippery solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils.

[0048] Tablet disintegrators include substances which swell when wetted to break up the tablet and release a compound, prodrug, isomer or pharmaceutically acceptable salt of this invention. They include starches, clays, celluloses, algins and gums. More particularly, corn and potato starches, methylcellulose, agar, bentonite, wood cellulose, powdered natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp and carboxymethylcellulose, for example, may be used as well as sodium lauryl sulfate.

[0049] Tablets are often coated with sugar as a flavor and sealant, or with film-forming protecting agents to modify the dissolution properties of the tablet. The compounds, prodrugs, isomers and pharmaceutically acceptable salts of this invention may also be formulated as chewable tablets, by using large amounts of pleasant-tasting substances such as mannitol in the formulation, as is now well-established in the art.

[0050] Where it is desired to administer a compound, prodrug, isomer or pharmaceutically acceptable salt of this invention as a suppository, any suitable base can be used. Cocoa butter is a traditional suppository base, which may be modified by the addition of waxes to raise its melting point. Water-miscible suppository bases comprising, particularly, polyethylene glycols of various molecular weights are also in wide use.

[0051] As discussed above, the effect of a compound, prodrug, isomer or pharmaceutically acceptable salt of this invention may be delayed or prolonged by proper formulation. For example, a slowly soluble pellet of a compound, prodrug, isomer or pharmaceutically acceptable salt of this invention may be prepared and incorporated in a tablet or capsule. The technique may be improved by making pellets of several different dissolution rates and filling capsules with a mixture of the pellets. Tablets or capsules may be coated with a film which resists dissolution for a predictable period of time. The parenteral preparations may also be made long-acting by dissolving or suspending a compound, prodrug, isomer or pharmaceutically acceptable salt of this invention, as the case may be, in oily or emulsified vehicles which allow it to disperse only slowly in the serum.

[0052] Generally, the compounds of this invention are administered in a pharmaceutically effective amount. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like, but in general will be about 0.01% to about 20% of the total weight of the composition.

[0053] The pharmaceutical compositions of this invention can be administered by any suitable routes including, by way of illustration, oral, topical, rectal, transdermal, subcutaneous, intravenous, intramuscular, intranasal, and the like. Depending on

the intended route of delivery, the compounds of this invention are preferably formulated as either oral, topical or injectable compositions.

[0054] Pharmaceutical compositions for oral administration can take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, such compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include prefilled, premeasured ampules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the nitrone compound is usually a minor component (from about 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

[0055] Liquid forms suitable for oral administration may include a suitable aqueous or nonaqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0056] Topical compositions are typically formulated as a topical ointment or cream containing the active ingredient(s), generally in an amount ranging from about 0.01 to about 20% by weight, preferably from about 0.1 to about 10% by weight, and more preferably from about 0.5 to about 15% by weight. When formulated as an ointment, the active ingredients will typically be combined with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with, for example, an oil-in-water cream base. Such topical formulations are well-known in the art and generally include additional ingredients to enhance the dermal penetration or stability of the active ingredients or the formulation. All such

known topical formulations and ingredients are included within the scope of this invention.

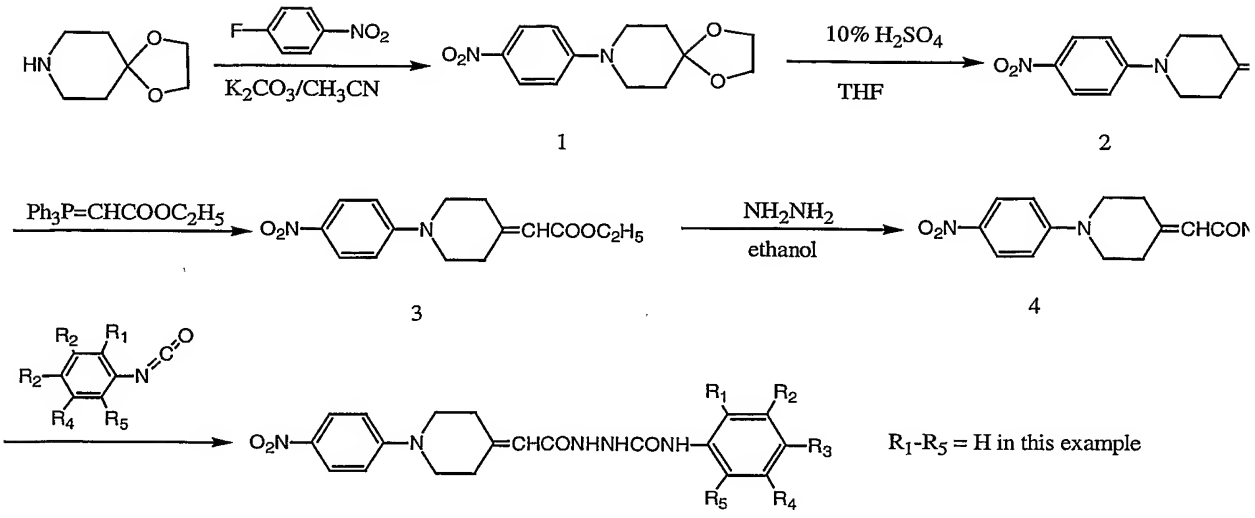
[0057] The compounds of this invention can also be administered by a transdermal device. Accordingly, topical administration can be accomplished using a patch either of the reservoir or porous membrane type or of a solid matrix variety.

[0058] Injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art. As before, the alkyl nitrone compound in such compositions is typically a minor component, often being from about 0.05 to 2% by weight with the remainder being the injectable carrier and the like.

[0059] The above-described components for orally and topically administrable or injectable compositions are merely representative. Other materials as well as processing techniques and the like are set forth in Part 8 of Remington's Pharmaceutical Sciences, 18th edition, 1990, Mack Publishing Company, Easton, Pennsylvania, 18042, which is incorporated herein by reference.

[0060] The compounds of this invention can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained release materials can be found in the incorporated materials in Remington's Pharmaceutical Sciences.

[0061] The following synthetic schemes and protocols may be used to synthesize compounds according to the invention.



Figure

[0062] The synthesis was carried out in 5 steps, as depicted the Figure above. In the first step (1), 2.78 g (20 mMol) of K_2CO_3 was added to a solution of 1,4-Dioxa-8-azaspiro[4.5]decane (2.86 g, 20 mMol) and 1-fluoro-4-nitrobenzene (1.41g, 10mMol) in 10ml acetonitrile. The resulting suspension was refluxed under nitrogen for 3 days, cooled to room temperature, and 50ml water was added and extracted with 300ml CH_2Cl_2 twice. The organic layers were combined, washed with brine, and dried over Na_2SO_4. Trituration with cold ethyl ether afforded 2.53g (96%) of 1 as a white solid. This compound was pure enough and no further purification was needed. ^1H NMR (CDCl3) δ 1.93 (triplet, 4H), 3.65 (triplet, 4H), 4.02 (singlet, 4H), 7.15 (doublet, 2H), 8.19 (doublet, 2H).

[0063] 1.69g (or 6.4 mMol) of 1 was dissolved in 20ml THF and 10ml 10% H_2SO_4 was added (1). This mixture was stirred at room temperature for 4 days, diluted with 30ml H_2O and extracted with 300 ml CH_2Cl_2. The organic part was washed with brine and dried. The solvent was removed to produce 1.32 g (94%) of 2, a slightly yellow solid. ^1H NMR (CDCl3) δ 2.68 (triplet, 4H), 3.91 (triplet, 4H), 6.98 (doublet, 2H), 8.22 (doublet 2H).

[0064] 0.66 g of 2 (3 mMol) and 2.1 g of $Ph_3P=CHCOOC_2H_5$ (6 mMol) were mixed and heated to 160°C overnight under N_2 (2). The reaction mixture was cooled to room temperature and purified by flash chromatography [hexane/ethyl acetate 7:3 (v/v), R_f 0.26] to give 0.65 g (76%) of 3. 1H NMR (CDCl3) δ 1.34 (triplet, 3H), 2.34 (broad,

2H), 3.11 (broad, 2H), 3.59 (broad, 2H), 3.89 (broad, 2H), 4.18 (multiplet, 2H), 5.71(br, 1H), 6.81 (triplet, 2H), 8.18 (triplet, 2H).

[0065] The mixture of 0.29 g of 3 (1 mMol) and 0.48 g anhydrous NH_2NH_2 (15 mMol) in 40 ml absolute ethanol was refluxed for 3 hours (3). After cooling to ambient temperature, the solution was concentrated in vacuo. The solid residue was crystallized from ethanol, affording compound 2.52 g (91%) of 4. ^{1}H NMR (DMSO) δ 2.22 (broad, 2H), 2.83 (broad, 2H), 3.60 (broad, 2H), 3.91 (broad, 2H), 5.68 (singlet, 1H), 7.02 (doublet, 2H), 8.05 (doublet, 2H)

[0066] In the final step, 0.5 mMol of the commercially available phenylisocyanate was added to a solution of 0.13 g 4 (0.5mmol) in 1ml dry CH_2Cl_2 (4), and stirred at room temperature for 2 hours. The final product was separated by filtration.

A1 ^{1}H NMR 1.10 (doublet, 6H), 2.16 (singlet, 3H), 2.26 (broad, 2H), 2.96 (broad, 2H), 3.34 (multiplet, 1H), 3.36 (broad, 2H), 3.92 (broad, 2H), 5.69 (singlet, 1H), 7.03 (multiplet, 3H), 7.13 (doublet, 2H), 7.87 (singlet, 1H), 8.05 (doublet, 2H), 9.78 (singlet, 1H). MS

A3 ^{1}H NMR 1.13 (doublet, 6H), 2.26 (broad, 2H), 2.97 (broad, 2H), 3.32 (multiplet, 1H), 3.64 (broad, 2H), 3.94 (broad, 2H), 5.71 (singlet, 1H), 6.96 (doublet, 2H), 7.12 (triplet, 2H), 7.26 (multiplet, 1H), 7.44 (multiplet, 1H), 8.11(doublet, 2H), 8.28 (singlet, 1H), 9.82 (singlet, 1H).

[0067] Combinatorial chemistry (Figure below) is used to design and synthesize four new classes of compounds (Schemes 1-4).

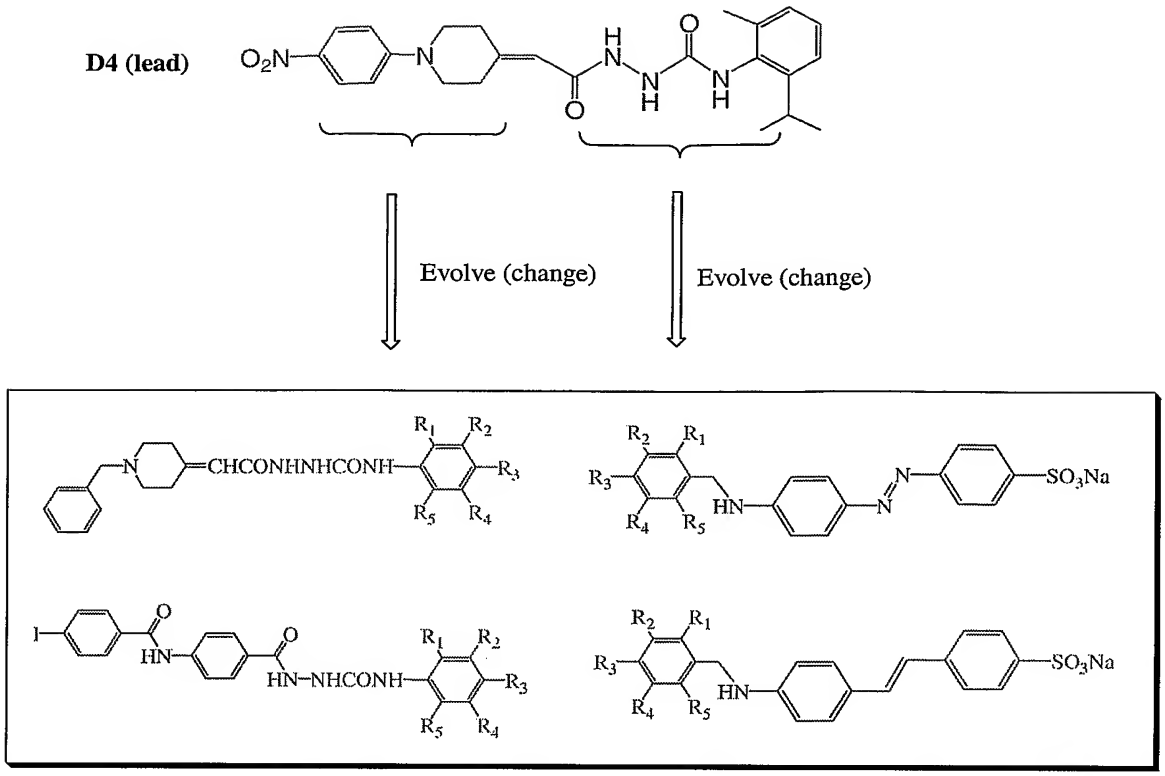
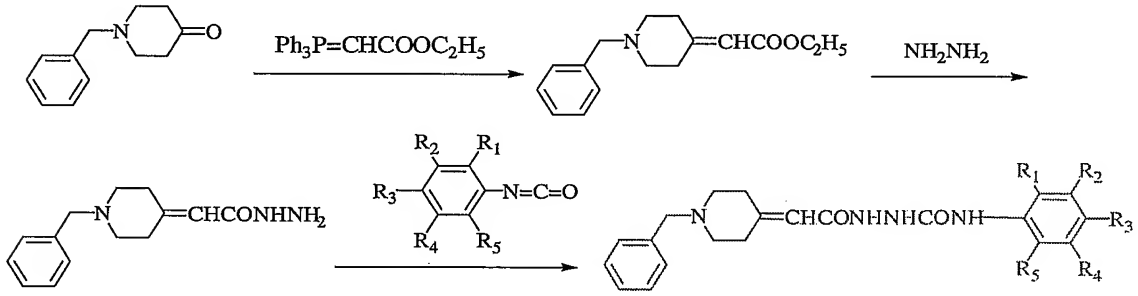
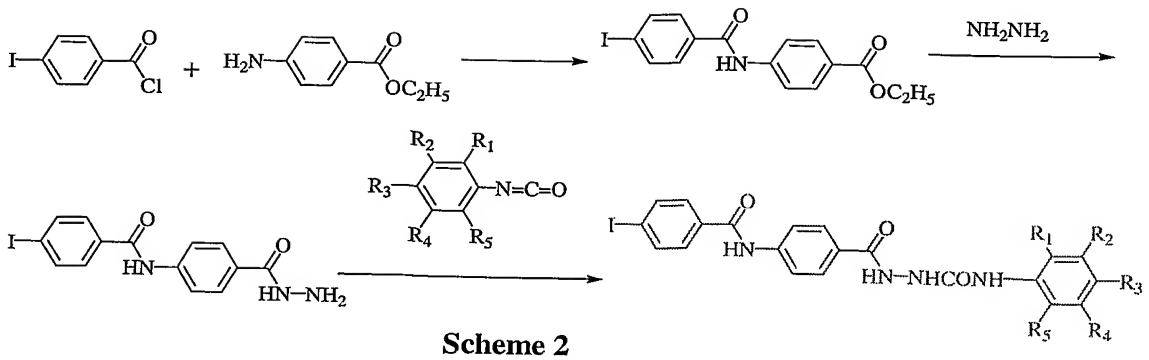


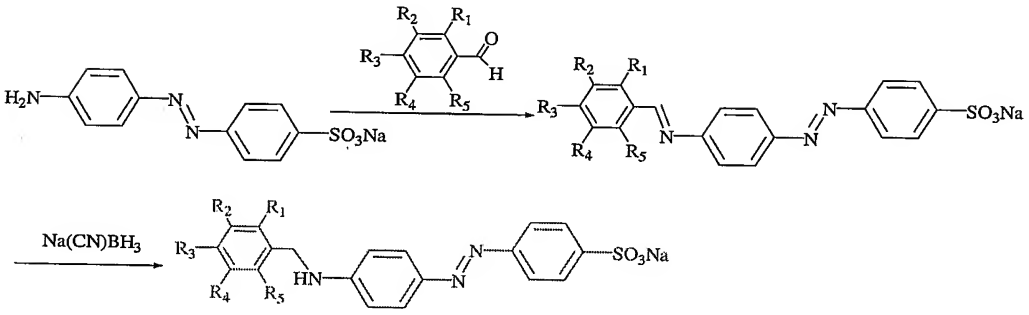
Figure: Combinatorial evolution of new compounds



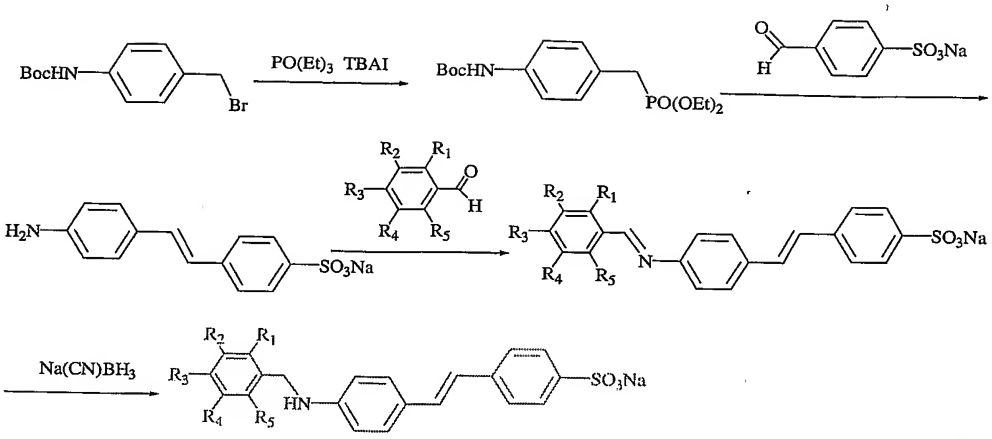
Scheme 1



Scheme 2



Scheme 3


BocHN
Br
PO(Et)3 TBAI
BocHN
PO(OEt)2
O
H
SO3Na
H2N
SO3Na
R2
R1
R3
R4
R5
O
H
N
Na(CN)BH3
HN

Scheme 4

[0068] While in no way intending to be bound by theory, in order to further illustrate and characterize the physiochemical and bioactive properties of the compounds and compositions of the invention, inventors provide the following non-limiting examples.

EXAMPLE 1

[0069] In order to study the inhibition of binding of [^{125}I]T3 to TRs in intact cells by the antagonist molecule Compound A, GH4 rat pituitary cells containing endogenous TRs (TRα1, TRβ1, and TRβ2) were grown in monolayer culture in DMEM medium containing 10% calf serum. Cells were dispersed by incubation in a buffered solution of EDTA and incubated at 37°C for 60 min in serum-free DMEM to lower endogenous levels of thyroid hormones. Aliquots containing approximately 1.5 million cells were collected by centrifugation at 1000 x g for 10 min and then suspended in 1mL of serum free medium containing 0.1nM [^{125}I]T3 and the indicated concentrations (see Figure 2) of unlabeled T3 or the antagonist candidate, Compound A. Following incubation at 37°C for 60 min, the cells were chilled in ice and then centrifuged at 4°C at 1000x g for 10 min. The samples were washed twice by re-suspension and vortexing with 1 ml of 50 mM Tris-HCl, pH 7.85, containing 1 mM $MgCl_2$ and 0.5% Triton X-100 and centrifugation at 1,000 x g for 10 min to isolate the nuclear fraction of the cells. The amount of [^{125}I]T3 retained in the resulting pellet of washed nuclei was determined using a Packard gamma spectrometer. The results are presented in Figure 2 as a percent of radioactivity retained in washed nuclei from cells incubated with 0.1 nM [^{125}I]T3 in the absence of unlabeled T3 or antagonist candidates. Each data point represents the average of duplicates, which generally varied by less than 5%.

EXAMPLE 2

[0070] Functional CAT assays were performed to compare the extent of inhibition of the T3 stimulation of CAT activity observed in the presence of the antagonist candidates. HeLa cells were innoculated at 50,000 cells per well in 24 well plates in DMEM containing 10% calf serum. The cells were transfected 5 hours later by calcium phosphate precipitation using 450ng of the T3 responsive ΔMTV-IR-CAT reporter and 250 ng of a vector expressing TRα. At the time of transfection, the cells also received 6nM T3 and the different concentrations of the antagonist candidates. Cells were harvested 40h after transfection and assayed for protein content and CAT activity. Results, shown in Figure 3, are expressed as the extent of inhibition of the T3 stimulation of CAT activity observed in the presence of the antagonist candidates.

Each data point reflects the average of triplicate samples which showed less than 10% variation.

EXAMPLE 3

[0071] A study to compare the T3-mediated co-activator recruitment to TR by A_1, A_3, and Compound A was conducted in vitro. Approximately 2.5-5 x 10^4 cpm of ^{35}S-labeled TRα (20 fmol) in 2 µl of lysate was incubated with 500 ng of GST fused to the receptor interaction region of the co-activator NRC (NRC15) immobilized on a glutathione-agarose beads. The samples were also incubated for 15 min at room temperature with of A_1 or A_3 or 5 µM of Compound A in binding buffer. The samples were then chilled on ice and incubated with 1nM T3 for an additional 60 min at 4°C. Control samples contained no T3 or antagonists, or received only T3. The beads were washed and the bound ^{35}S-TRα electrphoresed in a 10% SDS gel followed by analysis and quantitation of that amount of ^{35}S-TRα bound using a Molecular Dynamics Phosphorimager and ImageQuant software. The percent inhibition of T3-mediated binding of ^{35}S-TRα to GST-NRC15 by Compounds A, A_1 and A_3 was determined after subtracting the amount of ^{35}S-TRα bound to GST-NRC15 in the absence of T3. The results are shown in Figure 4 and show the inhibitory effect of the compounds as A_3>A_1>A, confirming the increased efficacy of compounds, synthesized from antagonists candidates chosen according to the Invention, whose selectivity is optimized as described herein.

EXAMPLE 4

Part one: UV-vis detection of A_3 in buffer

[0072] In order to detect the A_3 concentration in mice serum after the above study, standard UV absorption-concentration correlation was studied. Standard A_3 solution in buffer ranging from 100 µM to 1 nM with 10 time difference was prepared and their UV-vis absorption was measured. UV-vis can be used to detect 10 nM A_3 solution, and the detection limit can be enhanced by fluorescence. A_3 compound has Rf value of 0.3 when eluent is ethyl acetate in TLC study. A_3 compound has water solubility approximately of 10 µM and ethyl acetate can be used to extract A_3 out of aqueous phase. In addition, A_3 compound shows non-linear optical property.

[0073] The following references contain material associated with the field of the invention disclosed herein; however, no determination has been made with regard to the relevance or lack thereof of any of said references; moreover, no assertion that

any of said references is or is not relevant to the invention is intended. In the event that any of said references is actually found to contain material relevant to the present invention, such reference should be considered incorporated in its entirety into this specification.

1. DeGroot Ed. (1995), Saunders Endocrinology, 3rd ed., L.J.

2. Werner and Ingbar (1996) The Thyroid: a Fundamental and Clinical Test, Lippincott-Raven, 7th ed., L.E. Braverman and R.D. Utiger, eds.

3. Wilson J.D. Ed. (1998), Saunders Williams Textbook of Endocrinology

4. Yen, P.M. (2001) Physiol. Rev. 81, 1097-1142.

5. Evans RM. (1988) The steroid and thyroid hormone receptor superfamily. Science 240:889-95

6. Carson-Jurica MA, Schrader WT, O'Malley BW. (1990) Steroid receptor family: structure and functions. Endocr Rev. 11:201-20.

7. Chambon P. (1993) The molecular and genetic dissection of the retinoid signaling pathway. Gene.135:223-8.

8. Dees, E.C., Kennedy, M.J. (1998) Curr. Opin. Oncol. 10, 517-522.

9. Labrie F. (1993) Mechanism of action and pure antiandrogenic properties of flutamide. Cancer. 72:3816-27.

10. Olefsky JM, Saltiel AR (2000) PPAR gamma and the treatment of insulin resistance. Trends Endocrinol Metab. 11(9):362-8.

11. Forrest D, Erway LC, Ng L, Altschuler R, Curran T. (1996) Thyroid hormone receptor beta is essential for development of auditory function. Nat Genet. 13:354-7.

12. Weiss RE, Forrest D, Pohlenz J, Cua K, Curran T, Refetoff S. (1997) Thyrotropin regulation by thyroid hormone in thyroid hormone receptor beta-deficient mice. Endocrinology. 138:3624-9.

13. Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P, Vennstrom B. (1998) Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. EMBO J. 17:455-61.

14. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature. 389:753-8.

15. Moras D, Gronemeyer H. (1998) The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol., 10:384-91.

16. Weatherman RV, Fletterick RJ, Scanlan TS. (1999) Nuclear-receptor ligands and ligand-binding domains. Annu Rev Biochem. 68:559-81.

17. Bourguet W, Germain P, Gronemeyer H. (2000) Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci. 21:381-8

18. Pike AC, Brzozowski AM, Walton J, Hubbard RE, Thorsell AG, Li YL, Gustafsson JA, Carlquist M (2001) Structural insights into the mode of action of a pure antiestrogen. Structure (Camb) 9:145-53.

19. Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT, Parks DJ, Moore JT, Kliewer SA, Willson TM, Stimmel JB. (2002) Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha. Nature. 415:813-7.

20. Shiau, A. K., Barstad, D., Radek, J.T. Meyers, M.J. Nettles, K.W., Katzellenbogen, B.S., Katzellenbogen, J.A., Agard, D.A., Greene, G.L. (2002) Nat. Struc. Biol. 9, 359-364.

21. Shiau, A. K., Barstad, D., Loria, P. M., Cheng, L., Kushner, P. J., Agard, D. A., Greene, G. L. (1998) The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95, 927-937.

22. Pike, A.C., Brzozowski, A.M., Hubbard, R.E., Bonn, T., Thorsell, A.G., Engstrom, O., Ljunggren, J., Gustafsson, J.A., Carlquist, M. (1999) EMBO J. 18, 4608-18.

23. Bourguet, W., Vivat, V., Wurtz, J.M., Chambon, P., Gronemeyer, H., Moras, D. (2000) Mol. Cell 5, 289-98.

24. Schapira M, Raaka BM, Samuels HH, Abagyan R. (2000) Rational discovery of novel nuclear hormone receptor antagonists Proc Natl Acad Sci U S A. 97(3):1008-13.

25. Baxter JD, Goede P, Apriletti JW, West BL, Feng W, Mellstrom K, Fletterick RJ, Wagner RL, Kushner PJ, Ribeiro RC, Webb P, Scanlan TS, Nilsson S. (2002), Structure-based design and synthesis of a thyroid hormone receptor (TR) antagonist. Endocrinology; 143(2):517-24.

26. Darimont, B.D., Wagner, R.L., Apriletti, J.W., Stallcup, M.R., Kushner, P.J., Baxter, J.D., Fletterick, R.J., Yamamoto, K.R. (1998) Genes Dev. 12, 3343-56.

27. Molsoft LLC, ICM 2.8 manual, freely available online at www.molsoft.com (Molsoft, San Diego, CA).

28. Abagyan, R., Totrov, M. (1994) J. Mol. Biol. 235, 983-1002.

29. Totrov, M., Abagyan, R. (1997) Proteins, Suppl. 1, 215-20.

30. Totrov, M., Abagyan, R., (2001) in Drug-Receptor Thermodynamics: Introduction and Applications, ed., Raffa, R.B. (J. Wiley & Sons, Ltd.), pp. 603-624.

31. Abagyan, R., Totrov, M. (2001) Curr. Opin. Chem. Biol. 5, 375-82.

32. Raaka, B.M., Samuels, H.H. (1983) J. Biol. Chem. 258, 417-425.

33. Mahajan MA and Samuels HH. (2000). A new family of nuclear receptor coregulators that integrate nuclear receptor signaling through CREB-binding protein. Mol. Cell. Biol. 20; 5048-5063.

34. Li, D., Desai-Yajnik, V., Lo, E., Schapira, M., Abagyan, R., Samuels, H.H. (1999) Mol. Cell. Biol. 19, 7191-202.

35. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev.; 46(1-3):3-26.

36. Bourguet W., Ruff M., Chambon P., Gronemeyer H., Moras D. (1995) Nature 375, 377-82.

37. Strynadka N.C., Eisenstein M., Katchalski-Katzir E., Shoichet B.K., Kuntz I.D., Abagyan R., Totrov M., Janin J., Cherfils J., Zimmerman F., Olson A., Duncan B., Rao M., Jackson R., Sternberg M., James M.N. (1996) Nat Struct Biol. 3, 233-9.

38. Abagyan R., Batalov S., Cardozo T., Totrov M., Webber J., Zhou Y. (1997) Proteins., Suppl 1, 29-37.

39. Ajay A, Walters WP, Murcko MA. (1998) Can we learn to distinguish between "drug-like" and "nondrug-like" molecules? J Med Chem.; 41(18):3314-24.

40. Barratt MD, Rodford RA. (2001) The computational prediction of toxicity. Curr Opin Chem Biol. 5(4):383-8.

41. Casanova J, Horowitz ZD, Copp RP, McIntyre WR, Pascual A, Samuels HH.(1984) Photoaffinity labeling of thyroid hormone nuclear receptors. Influence of n-butyrate and analysis of the half-lives of the 57,000 and 47,000 molecular weight receptor forms J Biol Chem.; 259(19):12084-91

42. Egea PF, Mitschler A, Rochel N, Ruff M, Chambon P, Moras D. (2000) Crystal structure of the human RXRalpha ligand-binding domain bound to its natural ligand: 9-cis retinoic acid. EMBO J. Jun 1;19(11):2592-601.

43. Filikov AV, Mohan V, Vickers TA, Griffey RH, Cook PD, Abagyan RA, James TL. (2000) Identification of ligands for RNA targets via structure-based virtual screening: HIV-1 TAR J Comput Aided Mol Des. 14(6):593-610.

44. Greene N. (2002) Computer systems for the prediction of toxicity: an update. Adv Drug Deliv Rev.;54(3):417-31

45. Lazar MA (1993) Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr Rev. 14 (2):184-93

46. Macchia PE, Takeuchi Y, Kawai T, Cua K, Gauthier K, Chassande O, Seo H, Hayashi Y, Samarut J, Murata Y, Weiss RE, Refetoff S. (2001) Increased sensitivity

to thyroid hormone in mice with complete deficiency of thyroid hormone receptor alpha. Proc Natl Acad Sci U S A; 98(1):349-54.

47. McGovern SL, Caselli E, Grigorieff N, Shoichet BK. (2002) A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. J Med Chem. 45(8):1712-22.

48. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV.(1998) Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. Nature 395(6698):137-43.

49. Onate SA, Tsai SY, Tsai M-J, and O'Malley BW. (1995) Sequence and characterization of a coactivator of the steroid hormone receptor superfamily. Science 270; 1354-1357.

50. Oppenheimer JH, Koerner D, Schwartz HL, Surks MI (1972) J. clin. Endocrinol. Metab. 35:330-33

51. Oppenheimer, J.H. and H.H. Samuels. 1983. Molecular Basis of Thyroid Hormone Action. Academic Press, New York

52. Pohlenz J, Maqueem A, Cua K, Weiss RE, Van Sande J, Refetoff S. (1999) Improved radioimmunoassay for measurement of mouse thyrotropin in serum: strain differences in thyrotropin concentration and thyrotroph sensitivity to thyroid hormone. Thyroid.; 9(12):1265-71.

53. Powers CA, Mathur M, Raaka BM, Ron D, and Samuels HH. (1998) TLS (Translocated-in-Liposarcoma) is a high-affinity interactor for steroid, thyroid hormone, and retinoid receptors. Mol. Endocrinol. 12; 4-18.

54. Refetoff S. (1997) Resistance to thyroid hormone. Curr Ther Endocrinol Metab.; 6:132-4.

55. Sadowski J, Kubinyi H. (1998) A scoring scheme for discriminating between drugs and nondrugs. J Med Chem. 41(18):3325-9.

56. Samuels HH, Tsai JS. (1973) Thyroid hormone action in cell culture: demonstration of nuclear receptors in intact cells and isolated nuclei. Proc Natl Acad Sci U S A. 70:3488-92

57. Schapira M, Raaka BM, Samuels HH, Abagyan R. (2001) In silico discovery of novel Retinoic Acid Receptor agonist structures. BMC Struct Biol. 2001;1(1):1.

58. Steinmetz AC, Renaud JP, Moras D. (2001) Binding of ligands and activation of transcription by nuclear receptors. Annu Rev Biophys Biomol Struct. 30:329-59.

59. Takeuchi Y, Murata Y, Sadow P, Hayashi Y, Seo H, Xu J, O'Malley BW, Weiss RE, and Refetoff S. (2002) Steroid receptor coactivator-1 deficiency causes

variable alterations in the modulation of T(3)-regulated transcription of genes in vivo. Endocrinology 143; 1346-52.

60. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. (2002) Molecular properties that influence the oral bioavailability of drug candidates J. Med. Chem., 45 (12), 2615 -2623.

61. Viswanadhan VN, Balan C, Hulme C, Cheetham JC, Sun Y. (2002) Knowledge-based approaches in the design and selection of compound libraries for drug discovery. Curr Opin Drug Discov Devel.; 5(3):400-6.

62. Wagner, R. L., Darimont, B. D., Apriletti, J. W., Stallcup, M. R., Kushner, P. J., Baxter, J. D., Fletterick, R. J., Yamamoto, K. R.(1998) Structure and Specificity of Nuclear Receptor: Coactivator Interactions Genes Dev. 12, 3343.

63. Walters WP, Ajay, Murcko MA. (1999) Recognizing molecules with drug-like properties. Curr Opin Chem Biol. Aug;3(4):384-7.

64. Weiss RE, Refetoff S. (2000) Resistance to thyroid hormone. Rev Endocr Metab Disord. 1(1-2):97-108

65. Weiss RE, Chassande O, Koo EK, Macchia PE, Cua K, Samarut J, Refetoff S, Refetoff S. (2002) Thyroid function and effect of aging in combined hetero/homozygous mice deficient in thyroid hormone receptors alpha and beta genes. J Endocrinol.; 172(1):177-85.

66. Weiss RE, Xu J, Ning G, Pohlenz J, O'Malley BW, and Refetoff S. (1999). Mice deficient in the steroid receptor co-activator 1 (SRC-1) are resistant to thyroid hormone. Embo J.18; 1900-4.

67. Taylor, E. C.; Skotnicki, J. S. Synthesis. (1981), 8, 606.

68. Hu, Y.; Zorumski, C. F.; Covey, D. F. J. Org. Chem. (1995), 60, 3619.

69. Wang G.; Hollingsworth, R. I. Tetrahedron: Asymmetry. (1999), 10, 1895.

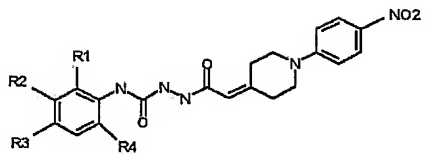
70. Plenat F.; Cassagne, M.; Cristau, H. J. Tetrahedron. (1995), 35, 9551

[0074] From the foregoing description, various modifications and changes in the compositions and methods of this invention will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

[0075] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

WHAT IS CLAIMED IS:

1. A compound capable of antagonizing the effects of thyroid hormone having the structure



wherein:

R_1 is $CH(CH_3)_2$, CH_2CH_3, CH_3, or H;

R_2 is CF_3, CH_3, F, or H;

R_3 is F, CH_3, OCH_3, CF_3, or H; and

R_4 is CH_3, OCH_3, or H

provided that

when R_2 is CF_3, R_1 and R_3 are H;

when R_1 is $CH(CH_3)_2$, R_2 and R_3 are H and R_4 is either CH_3 or H;

when R_1 is CH_2CH_3, R_2, R_3, and R_4 are H;

when R_2 is CH_3, R_1 and R_3 are H and R_4 is OCH_3;

when R_2 is F, R_3 is also F and R_1 and R_4 are H;

when R_3 is F, R_2 is also F and R_1 and R_4 are H;

when R_1 is CH_3, R_3 and R_4 are also CH_3 and R_2 is H;

when R_3 is OCH_3, R_1, R_2, and R_4 are H;

when R_3 is CF_3, R_1, R_2, and R_4 are H.

2. A compound according to claim 1 wherein R_1 is $CH(CH_3)_2$ and R_2, R_3, and R_4 are H.

3. A compound according to claim 1 wherein R_1 is CH_2CH_3 and R_2, R_3, and R_4 are H.

4. A compound according to claim 1 wherein R_1 is $CH(CH_3)_2$, R_4 is CH_3, and R_2 and R_3 are H.

5. A compound according to claim 1 wherein R_2 is CH_3, R_4 is OCH_3, and R_1 and R_3 are H.

6. A compound according to claim 1 wherein R_2 is F, R_3 is F, and R_1 and R_4 are H.

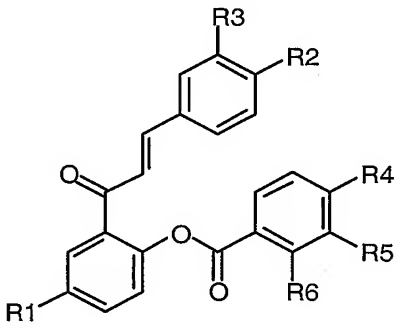
7. A compound according to claim 1 wherein R_1, R_3, and R_4 are CH_3, and R_2 is H.

8. A compound according to claim 1 wherein R_1, R_3, and R_4 are CH_3, and R_2 is H.

9. A compound according to claim 1 wherein R_3 is OCH_3, and R_1, R_2, and R_4 are H.

10. A compound according to claim 1 wherein R_3 is CF_3, and R_1, R_2, and R_4 are H.

11. A compound capable of antagonizing the effects of thyroid hormone having the structure



wherein

R1 = F, R2 = Cl, R3 = H, R4=H, R5=NO_2, R6=H

R1 = F, R2 = Cl, R3 = H, R4=H, R5=H, R6=OCH_3

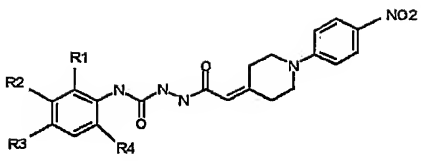
R1 = F, R2 = OCH_3, R3 = OCH_3, R4=H, R5=NO_2, R6=H

R1 = F, R2 = Cl, R3 = H, R4=NO_2, R5=H, R6=H

R1 = CH_3, R2 = F, R3 = H, R4=H, R5= OCH_3, R6=H

12. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound according either of claims 1 or 11 and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition comprising a pharmaceutically effective amount of a compiund having the structure



wherein

R_1 is $CH(CH_3)_2$, CH_2CH_3, CH_3, or H;

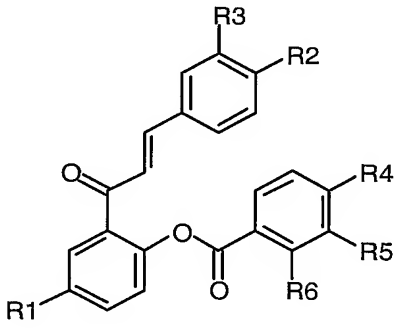
R_2 is CF_3, CH_3, F, or H;

R_3 is F, CH_3, OCH_3, CF_3, or H; and

R_4 is CH_3, OCH_3, or H,

and a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound having the structure



wherein:

R1 is F;

R2 may be Cl, OCH_3 or F;

R3 may be H or OCH_3;

R4 may be H or NO_3;

R5 may be H, OCH_3 or NO_2; and

R6 may be H or OCH_3.

and a pharmaceutically acceptable carrier.

15. A pharmaceutical composition according to any of claims 12, 13, or 14, wherein the composition is effective as an antagonist of thyroid hormone for modulation of a condition in a human characterized by overproduction of thyroid hormone to a degree detrimental to health.

16. A method of controlling thyroid hormone receptor activity comprising the in vivo administration of a compound according to either of claims 1 or 11.

17. A method of controlling thyroid hormone receptor activity comprising the in vivo administration of a therapeutically effective amount of a composition according to any of claims 12, 13, or 14.

18. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising administering a therapeutically effective amount of a composition according to any of claims 12, 13, or 14.

19. The method of any of claims 16-18 wherein the condition is selected from the group consisting of hyperthyroidism and thyrotoxicosis.

20. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising administering a pharmaceutically effective amount of a compound according to either of claims 1 or 11.

21. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising

i) administering a pharmaceutically effective amount of a compound according to either of claims 1 or 11; and

ii) administering a treatment selected from the group consisting of administering a pharmaceutical agent capable of inhibiting the synthesis of thyroid hormone, destruction of thyroid tissue by radioactive iodine, and surgical removal of thyroid tissue.

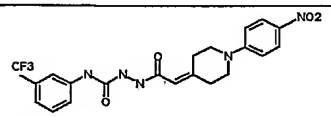
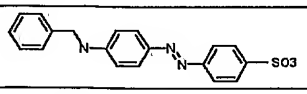
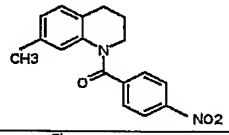
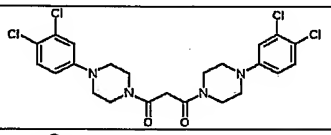
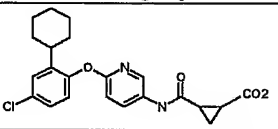
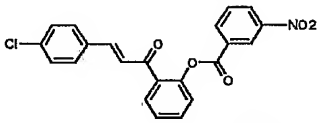
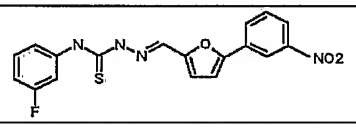
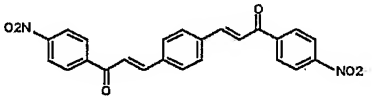
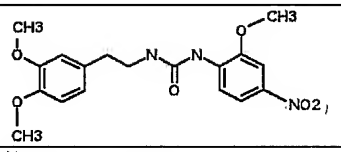
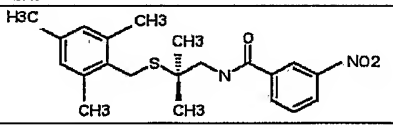
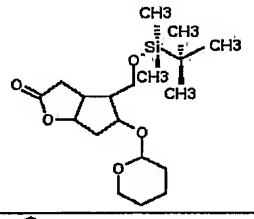
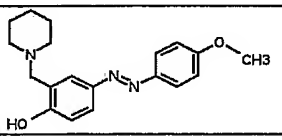
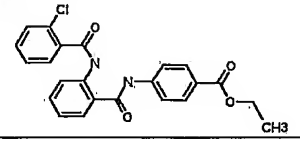
22. The method of either of claims 20 or 21, wherein the condition is selected from the group consisting of hyperthyroidism and thyrotoxicosis.

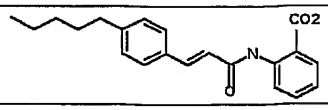
23. The method of claim 22 wherein the condition is selected from the group consisting of hyperthyroidism and thyrotoxicosis.

24. A method for preparing compounds useful for the treatment of a human having a condition characterized by overproduction of thyroid hormone, said method comprising the steps of:

i) selecting a compound selected from the group consisting of

Name	Structure

Compound A	
Compound B	
Compound C	
Compound D	
Compound E	
Compound F	
Compound G	
Compound H	
Compound I	
Compound J	
Compound K	
Compound L	
Compound M	

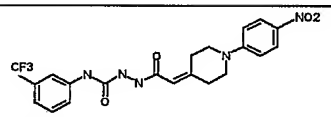
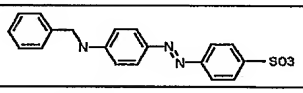
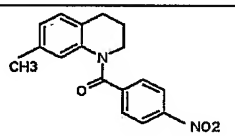
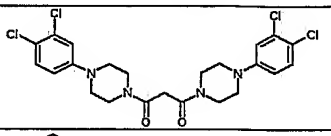
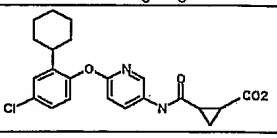
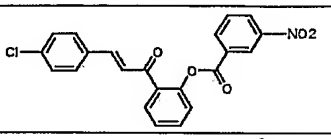
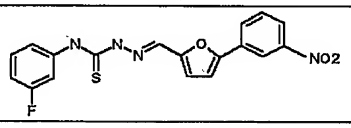
Compound N	

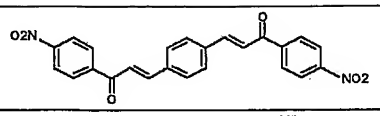
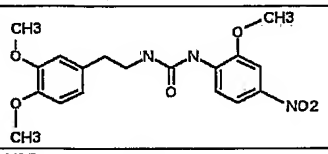
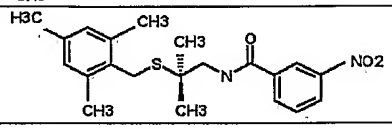
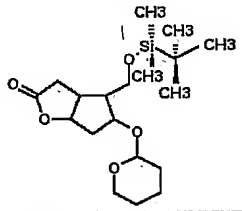
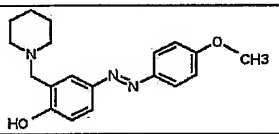
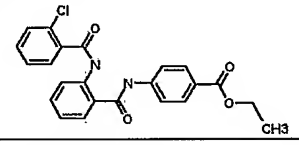
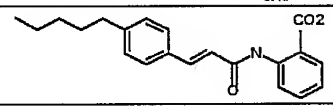
ii) generating a virtual library of derivatives of the compound chosen in step i);
iii) screening said library in silico;
iv) chemically synthesizing at least one compound screened in iii); and
v) testing in vitro at least one compound synthesized in iv).

25. The method of claim 24 further comprising the additional step of testing in vivo at least one compound chemically synthesized in iv).

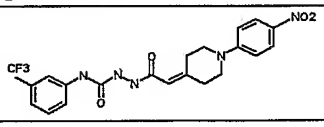
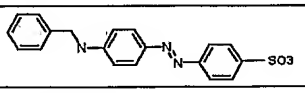
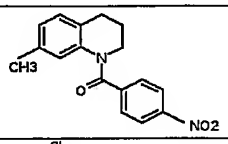
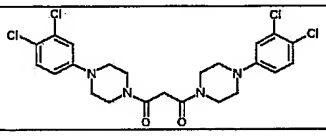
26. A pharmaceutical composition comprising a pharmaceutically effective amount of one or more compounds according to either of claims 1 or 11 and a pharmaceutically acceptable carrier.

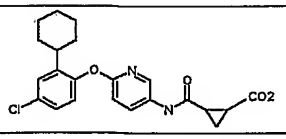
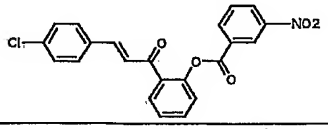
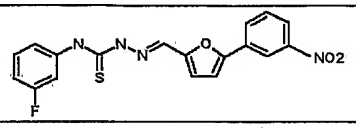
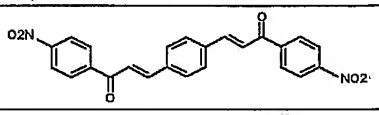
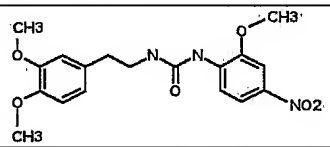
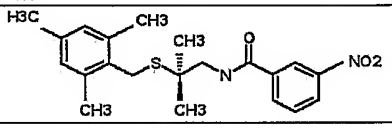
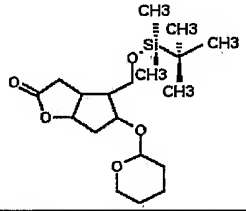
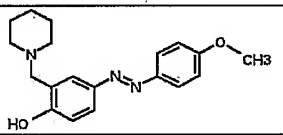
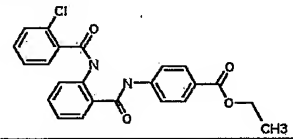
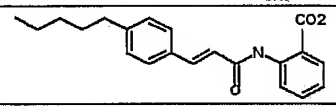
27. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising administering a pharmaceutically effective amount of at least one compound selected from the group consisting of:

Name	Structure
Compound A	
Compound B	
Compound C	
Compound D	
Compound E	
Compound F	
Compound G	

Compound H	
Compound I	
Compound J	
Compound K	
Compound L	
Compound M	
Compound N	

28. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising administering a pharmaceutically effective amount of a pharmaceutical composition containing, as an active ingredient, a pharmaceutically effective amount of at least one compound selected from the group consisting of:

Name	Structure
Compound A	
Compound B	
Compound C	
Compound D	

Compound E	
Compound F	
Compound G	
Compound H	
Compound I	
Compound J	
Compound K	
Compound L	
Compound M	
Compound N	

29. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising administering a pharmaceutically effective amount of at least one compound according to claim 27.

30. A method of treating a human for a condition characterized by overproduction of thyroid hormone comprising:

i) administering a pharmaceutically effective amount of one or more compounds according to either of claims 1 or 11; and

ii) administering a treatment selected from the group consisting of administering a pharmaceutical agent capable of inhibiting the synthesis of thyroid hormone, destruction of thyroid tissue by radioactive iodine, and surgical removal of thyroid tissue.

31. A method of controlling thyroid hormone receptor activity comprising the in vivo administration of one or more compounds according to claim 1.

32. A method of controlling thyroid hormone receptor activity comprising the in vivo administration of one or more compounds according to claim 11.

33. Use of a compound according to either of claims 1 or 11 for the preparation of a composition for the treatment of aberrant thyroid activity in a mammal.

34. Use of a composition according to any of claims 12-14 for the treatment of aberrant thyroid activity in a mammal.

35. Use according to either of claims 33 or 34 wherein the mammal is a human.

36. Use according to any of claims 33-35 wherein said aberrant thyroid activity comprises overproduction of thyroid hormone.

37. Use according to claim 36 wherein said overproduction of thyroid hormone is selected from the group consisting of hyperthyroidism and thyrotoxicosis.

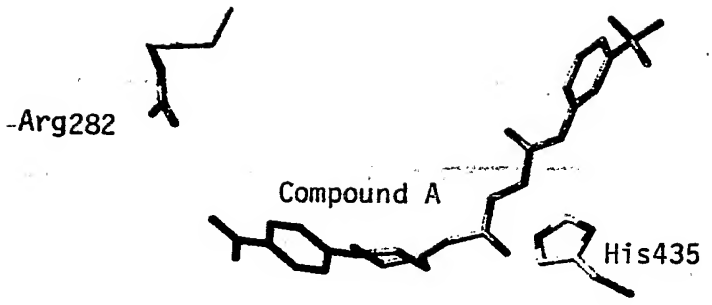


FIGURE 1A

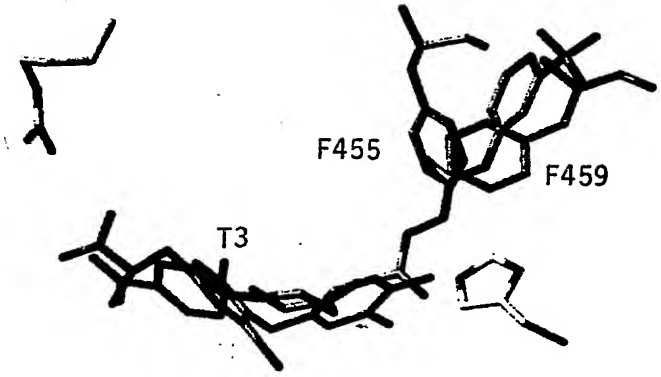


FIGURE 1B

FIGURE 2

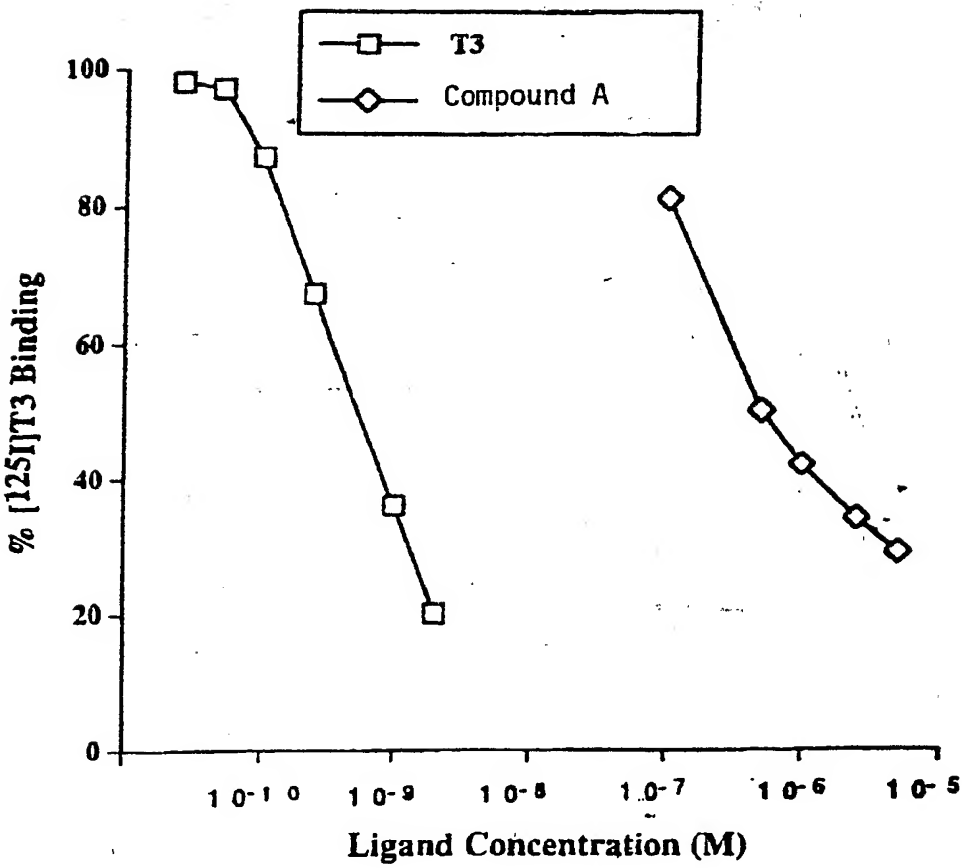

T3
Compound A
100
80
60
40
20
0
% [125]T3 Binding
10-10
10-9
10-8
10-7
10-6
10-5
Ligand Concentration (M)

FIGURE 3

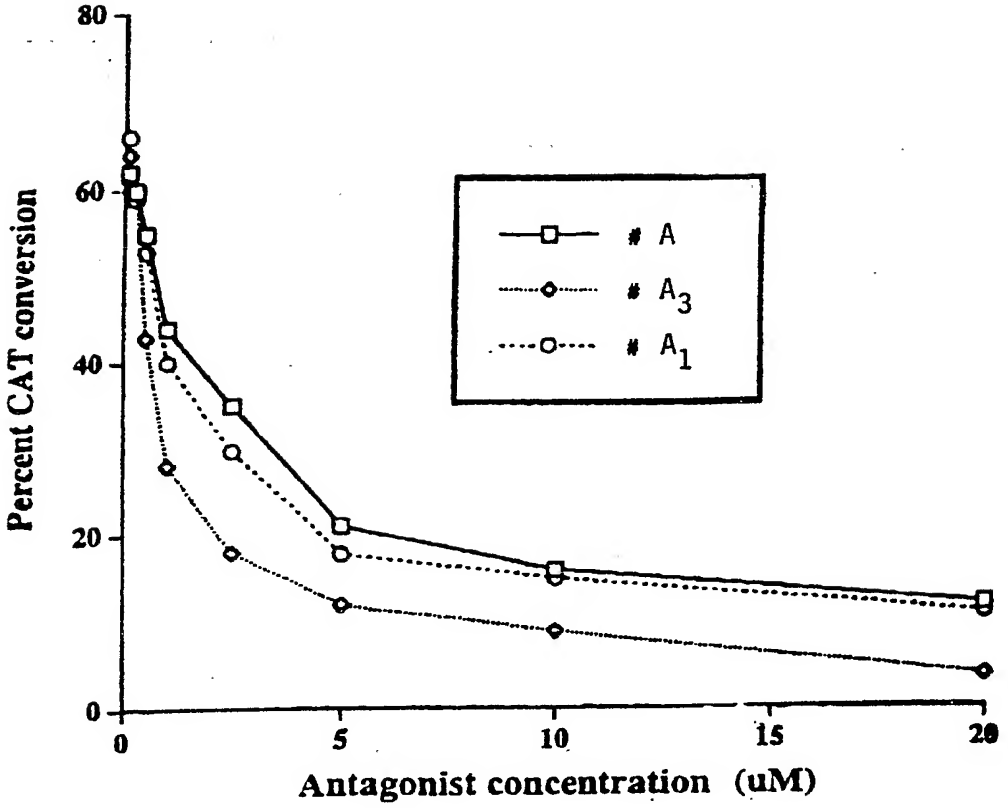
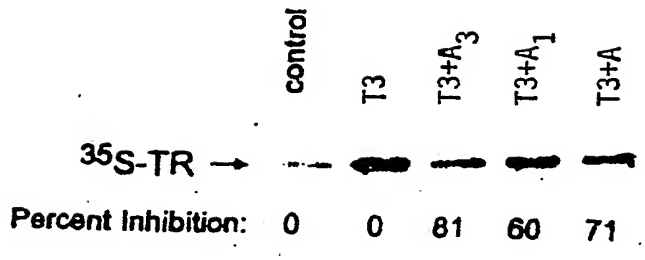

80
60
40
20
0
Percent CAT conversion
0
5
10
15
20
Antagonist concentration (uM)
A
A3
A1

FIGURE 4


control
T3
T3+A3
T3+A1
T3+A
35S-TR
Percent Inhibition: 0 0 81 60 71

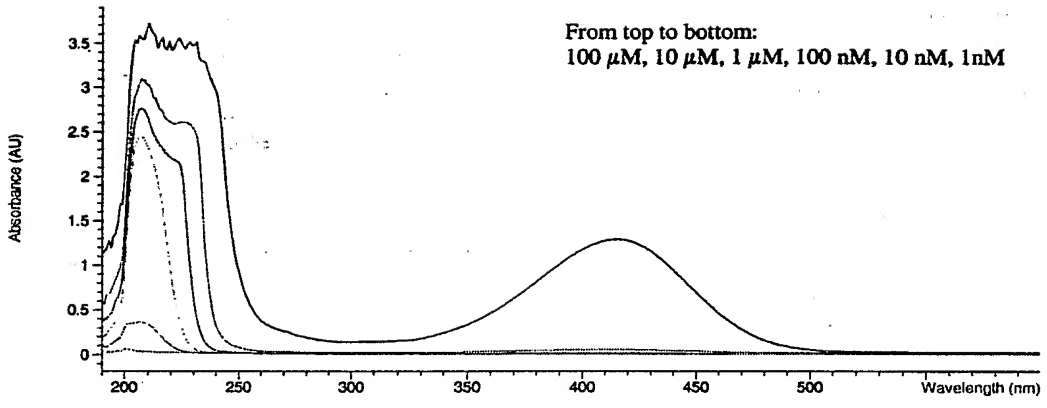

From top to bottom:
100 μM, 10 μM, 1 μM, 100 nM, 10 nM, 1nM
Absorbance (AU)
3.5
3
2.5
2
1.5
1
0.5
0
200
250
300
350
400
450
500
Wavelength (nm)

FIGURE 5